Isobutylmethylxanthine Fails to Stimulate Chloride Secretion in Cystic Fibrosis Airway Epithelia

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It has been proposed that a combination of an activated adenylyl cyclase and a high concentration of a phosphodiesterase inhibitor (isobutylmethylxanthine [IBMX], 5 mM) stimulates Cl^- secretion mediated by the heterologously expressed cystic fibrosis transmembrane regulator protein carrying the most common cystic fibrosis (CF) mutation (ΔF508). We tested whether Cl^- secretion could be stimulated by this protocol *in vitro* and *in vivo* in CF airway epithelia expressing endogenous ΔF508 CFTR protein. In cultured airway preparations, forskolin (a direct adenylyl cyclase activator) stimulated Cl^- secretion in amiloride-pretreated normal (ΔI_{sc} = 7.1 ± 1.7 $\mu A \cdot cm^{-2}$) but not CF tissues (ΔI_{sc} = −0.2 ± 0.1 $\mu A \cdot cm^{-2}$). Unexpectedly, IBMX partially inhibited the forskolin-induced Cl^- secretion in normal tissues; IBMX addition had no effect on CF tissues. Direct measurements of cell cAMP concentrations revealed that 0.1 mM IBMX and forskolin produced the maximum levels of cell cAMP levels attainable with this drug combination, and 5 mM IBMX was without further effect. The combination of forskolin (10^{-5} M) and isoproterenol, an adenylyl cyclase activator (10^{-5} M), produced approximately 3 times higher levels of cAMP than forskolin/IBMX but also did not induce Cl^- secretion in CF tissues. Studies of Cl^- secretion *in vivo*, assessed by the transepithelial electric potential difference (PD), showed that isoproterenol (10^{-5} M) stimulated Cl^- secretion (ΔPD = −16.3 ± 4.3 mV; n = 4) in nasal epithelia of normal subjects but not in CF patients homozygous for the ΔF508 mutation (ΔPD = −2.6 ± 1.9 mV; n = 5). No additive effects of IBMX (5 mM) were observed in either group. We conclude that combinations of adenylyl cyclase activators and IBMX are not effective in initiating Cl^- secretion in CF airway epithelia.

Cystic fibrosis (CF) is expressed in the lung as a chronic bacterial infection of the airways that leads to diffuse bronchiectasis (1). Although the detailed pathophysiology of CF lung disease is not understood, it is likely that abnormal electrolyte transport is a primary contributor to airways dysfunction in CF. A reduced capacity to secrete Cl^- ions toward the airway lumen in response to agents that regulate cell cyclic adenosine monophosphate (cAMP) levels, e.g., β-agonists (2–4), and hyperabsorption of Na^+ ions from the airway lumen to the blood compartment (5–8) have both been reported as electrolyte transport abnormalities in CF airways.

Pharmacologic therapies designed to modify abnormal electrolyte transport in the CF lung may prove beneficial. The Na^+ channel blocker, amiloride, provides partial protection of the lung from disease-induced decline in airflow obstruction (9). Further therapeutic benefit may be expected from activating the defective Cl^- secretory path. Direct pharmacologic studies of this problem have been made possible by the cloning of the CF gene (10). Using recombinant DNA technology, the protein product of this gene, the "cystic fibrosis transmembrane regulator" (CFTR), has been expressed in heterologous cell systems, e.g., *Xenopus* oocytes (11). Initial reports suggest that CFTR is the cAMP-dependent Cl^- channel itself (12, 13). In the recent studies of Drumm and colleagues (11), data were obtained indicating that frog oocytes expressing CFTR containing the common phenylalanine deletion CF mutation ΔF508 could be stimulated to generate a Cl^- current when exposed to a combination of a direct activator of adenylyl cyclase (forskolin) and high concentrations (5 mM) of the phosphodiesterase inhibitor isobutylmethylxanthine (IBMX). These researchers speculated that partial Cl^- channel function of the mutated CFTR (ΔF508) could be achieved by a high degree of cAMP-dependent phosphorylation of the CFTR protein.

In the present study, we attempted to induce Cl^- secretion in CF airway epithelia by raising intracellular cAMP to high levels using combinations of adenylyl cyclase activators and a high concentration of IBMX. *In vitro* experiments employing cultured cell preparations and *in vivo* bioelectric

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measurements of the apical membrane Cl^- conductance of airway epithelia were utilized.

Materials and Methods

Subjects

For the *in vitro* studies, nasal or bronchial specimens were obtained from six CF patients during surgery for nasal obstruction or lung transplantation (3 males, 3 females; mean age, 20.4 ± 3.4 yr). Five CF patients were genotyped, as follows: four were ΔF508/ΔF508; one ΔF508/unknown. Specimens from normal subjects were obtained from nasal reconstructive surgery (13 men, 4 women; mean age, 43.8 ± 4.6 yr). All normal subjects had no family history of CF.

For the *in vivo* studies, five CF patients, homozygous for the ΔF508 mutation, were studied (3 men, 2 women; mean age, 29.4 ± 1.6 yr). Normal subjects for the *in vivo* electric potential difference (PD) studies were age-matched for the CF subjects (1 man; 3 women; mean age, 27.3 ± 1.3 yr). All were free of disease, were using no medications, and had no family history of CF. All studies were approved by the University of North Carolina Committee on the Rights of Human Subjects.

Cell Culture

Cell culture techniques have been previously described (14). In brief, cells were disaggregated from resected airway specimens (4°C, 24 to 48 h; protease XIV; Sigma Chemical Co., St. Louis, MO), concentrated, and plated on collagen matrix supports (CMS), 3.0 or 4.5 mm in diameter (two to six preparations per patient) for bioelectric studies. Transwell-Cols (1.2 cm; Costar; Cambridge, MA) were employed in studies of cAMP metabolism. Cells were maintained in F-12 media supplemented with insulin (10 μg/ml), endothelial cell growth substance (7.5 μg/ml), transferrin (5 μg/ml), T_3 (10^{-9} m), and hydrocortisone (5×10^{-9} M). After 5 days in culture, the media was supplemented (1:1) with 3T3 cell conditioned media (15). Cells plated on the CMS were monitored daily for confluence and development of transepithelial electric PD.

Measurement of cAMP Accumulation

cAMP accumulation was monitored essentially as described previously (14, 16). Briefly, 0.7 and 0.3 ml of Hepes-buffered (20 mM, pH 7.5) Eagle's modified essential medium (MEM) containing 2 to 3 μCi/ml of [^{3}H]adenine were added to the serosal and mucosal side of the epithelial sheets, respectively, and the cells were incubated for 3 h at 37°C. The medium was aspirated, the cells were washed twice with Hepes-MEM, and fresh aliquots, same volume as above, of Hepes-MEM containing pertinent drugs were added. The reactions were carried out at 37°C and were terminated at the indicated times by aspiration of the medium followed by the immediate bilateral addition of 5% trichloroacetic acid containing 0.5 mM cAMP. [^{3}H]cAMP was separated from [^{3}H]ATP by sequential passage of the trichloroacetic acid extract over Dowex and alumina columns. Aliquots of the cAMP fractions were assayed spectrophotometrically at 259 nm to correct for recovery of cAMP. Experiments were carried out with triplicate samples that differed < 10% from the mean. The results are expressed as the percent of conversion of [^{3}H]ATP to [^{3}H]cAMP.

Bioelectric Characterization of Cl^- Transport in Cultured Airway Epithelia

Cell preparations were mounted in Ussing chambers and bathed in a Krebs bicarbonate Ringers solution that was gassed (95% O_2/5% CO_2) and warmed (37°C). The preparations were voltage-clamped to record the short-circuit current (I_{sc}), a measure of active ion transport rates. Transepithelial voltage pulses (± 10 mV) were generated intermittently (every 6 s) so that transepithelial resistance could be calculated. I_{sc} and the voltage pulses were recorded on a strip chart recorder. After steady-state I_{sc} was achieved, drugs were added cumulatively. Amiloride (10^{-4} M) was added to the apical bathing solution to convert the tissues from the Na^+-absorptive to the Cl^--secretory mode (17). Forskolin, at a maximally effective concentration (10^{-5} M) as determined in preliminary studies, was added bilaterally and I_{sc} was measured for 10 min. Preliminary studies in normal tissues showed that IBMX was equally effective when applied to the apical or basolateral surface, and the effects of exposure of each surface to maximal IBMX concentration were not additive. Because of effects on ATP responses when applied to the apical surface (see below), IBMX was usually applied to the basolateral surface and I_{sc} was monitored for an additional 10 min. In some experiments, a combination of isoproterenol (10^{-5} M) and forskolin (10^{-5} M) was added after amiloride with and without IBMX (0.1 mM) pretreatment. ATP (10^{-4} M) was added to the apical surface at the end of each protocol.

In Vivo Transepithelial Electric PD

The *in vivo* PD technique previously reported by Knowles and co-workers (18, 19) was employed. In brief, the transepithelial PD was measured between a Ringer's-filled exploring bridge (PE 50 tubing) positioned on the nasal mucosa and a reference bridge (Ringer 4% agar in a 21-gauge needle) positioned underneath the skin of the forearm. The PD was measured by a voltmeter (University of North Carolina Department of Electronics) and recorded on the strip chart. For delivery of drugs and solutions of different ionic composition, a second bridge (PE 50 tubing) positioned adjacent to the exploring bridge was utilized to perfuse solutions at the site of the PD measurement at the rate of 5 ml/min. The typical protocol involved the following sequential measurements: (*1*) steady-state PD (< 5% change over 30 s) during superfusion of either Cl^--free (gluconate replacement) Ringer's or low Cl^-, raised K^+ solution containing amiloride (10^{-4} M); (*2*) addition of the β-agonist isoproterenol (10^{-5} M) and amiloride on the mucosal surface for 3 min; (*3*) superfusion of IBMX (5 mM), isoproterenol (10^{-5} M), and amiloride for 5 min; (*4*) superfusion of ATP (10^{-4} M) plus isoproterenol, IBMX, and amiloride onto the mucosal surface for 2 min.

Solutions and Drugs

A Krebs Ringer's bicarbonate solution was utilized for *in vitro* studies of cultured monolayers (20, 21). In some *in vitro* studies, the apical solution of the culture was bathed by solutions containing low Cl^- (3 mM Cl^-, Cl^- replaced by gluconate), raised K^+ (100 mM, replacing Na^+), and amiloride (10^{-4} M). Similar solutions and Cl^--free only (gluconate

replacement [22]) were utilized for the *in vivo* experiments, except that 25 mM Hepes was utilized to buffer the pH of the high K^+, low Cl^- Ringer's solution because of the inability to keep the solution adequately gassed with 5% CO_2 throughout the perfusion tubing. Forskolin (Sigma) dissolved in a 10^{-2} M stock of ethanol vehicle, isoproterenol (Sigma) dissolved in 10^{-2} M stock in appropriate vehicle, and ATP (Boehringer Mannheim, Indianapolis, IN) dissolved in Ringer's as a 10^{-2} M stock were utilized. IBMX (Sigma) was added to the Ussing chamber as a dry powder, or as a suspension in Ringer's (50 mM stock), or after generation of a 200 mM stock solution (300 mM sucrose, pH 8.5 solution). No differences in bioelectric effects in normal tissues were noted among the different delivery modes. For cAMP studies, IBMX was dissolved in 0.1 N NaOH as a 50 mM stock. Solutions containing the desired final concentration of IBMX were adjusted to pH 7.4 with HCl before their addition to the tissues.

Statistics

For the *in vitro* studies, the maximum change in I_{sc} induced by an agonist was compared with baseline. Significance of changes was estimated by utilizing paired *t* tests for group data (4 to 15 per group). To estimate the difference in responses to agonists between CF and normal preparations, changes induced by agonists for the two groups were compared by unpaired *t* tests. For the *in vivo* PD responses, changes induced by agonists at different time points were compared with basal PDs within a group by paired *t* tests and the changes between the CF and normal groups compared by unpaired *t* tests. Unless otherwise stated, all values are mean ± SEM, with the number of tissues shown in parentheses.

Results

Figure 1 shows tracings of the responses of normal and CF cell culture preparations, pretreated with amiloride to generate Cl^- secretion, to the sequential addition of forskolin (10^{-5} M) and IBMX (5 mM). In response to forskolin, the normal tissue shows an increase in the I_{sc}, an index of Cl^- secretion, followed by inhibition of I_{sc} in response to addition of IBMX. The CF tissue exhibits a smaller residual I_{sc} after amiloride, consistent with the defective basal Cl^- conductance that limits the capacity to generate a Cl^- secretory current (2–4, 23). Neither forskolin alone nor forskolin combined with IBMX induces a Cl^- secretory current in the CF preparation. The mean data for a series of tissues exposed to these maneuvers are shown in Figure 2A. Forskolin routinely induces an increase in Cl^- secretion in normal tissues followed by a modest inhibition of the forskolin-stimulated Cl^- current with the subsequent addition of IBMX. In contrast, CF tissues respond neither to forskolin nor to the combination of forskolin and IBMX.

We used ion substitution maneuvers to increase the sensitivity of the *in vitro* system to small effects of the IBMX on the Cl^- secretory path (Figure 2B). In these studies, the apical side was bathed with low Cl^-, raised K^+ amiloride (10^{-4} M)-containing solutions to generate a much larger electrochemical driving force selectively favoring Cl^- secretion than occurs after amiloride treatment alone (17, 24).

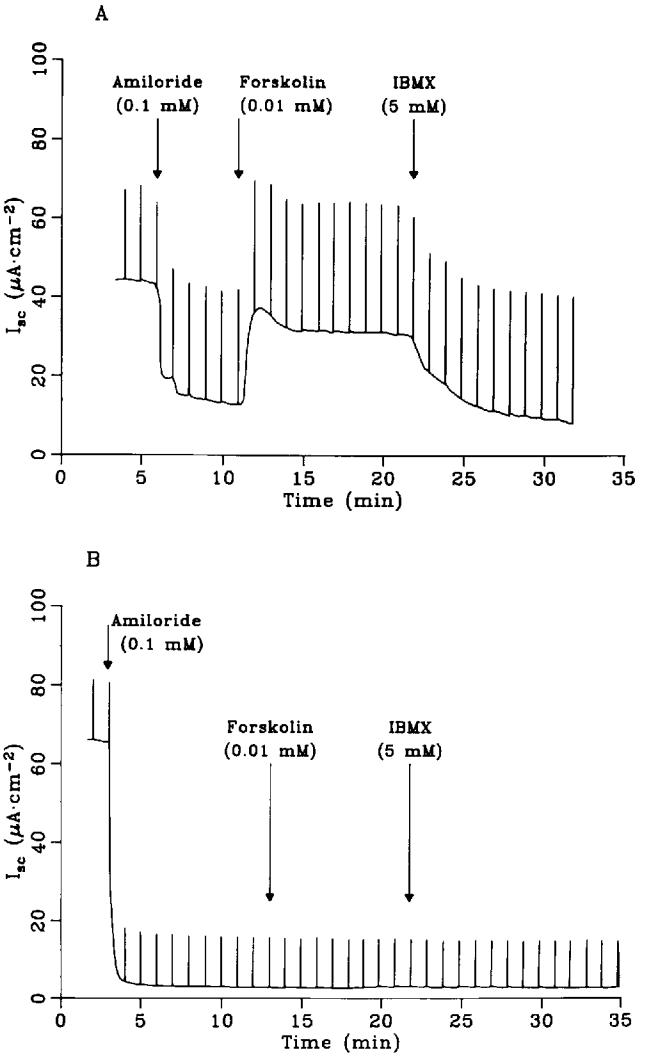

Figure 1. Cl^- secretory responses (I_{sc}) of amiloride (10^{-4} M)-pretreated cultured normal (A) and CF (B) airway epithelial preparations to cumulative addition of forskolin (10^{-5} M) and IBMX (5 mM).

Under these conditions, forskolin induced a large increase in I_{sc} in normal tissues, whereas an inhibition of this current was observed with addition of 5 mM IBMX (Figure 2B). There was no effect of forskolin or IBMX (5 mM) in forskolin-pretreated tissues under these conditions in CF tissues.

Because CF tissues failed to secrete Cl^- ions in response to forskolin and IBMX, we tested the response of CF preparations to a Cl^- secretagogue of a different class, ATP (25). In preliminary studies, we noted that the presence of 5 mM IBMX bilaterally in the bathing solutions resulted in a smaller response to mucosal ATP than typically observed in normal or CF airway epithelia. In both normal (n = 9) and CF (n = 5) tissues, the response to apical ATP was blunted in the presence of apical IBMX (ΔI_{sc} = 14.4 ± 2.1 μA·cm^{-2}, normal; ΔI_{sc} 19.7 ± 8.9 μA·cm^{-2}, CF) as compared with basolateral IBMX (ΔI_{sc} = 28.4 ± 5.9 μA·cm^{-2}, normal; ΔI_{sc} = 29.1 ± 8.6 μA·cm^{-2}, CF; $P < 0.05$). Thus, it appears that IBMX may be a relatively weak antagonist for

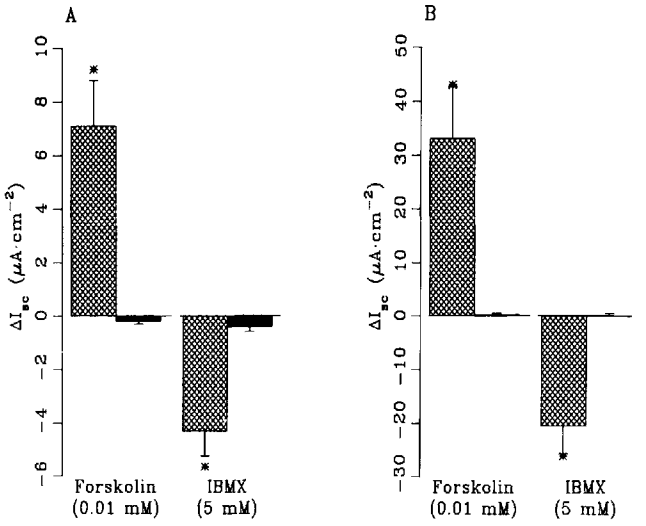

Figure 2. Mean Cl^- secretory response (ΔI_{sc}) of amiloride (10^{-4} M)-pretreated cultured normal (*hatched bars*) and CF (*solid bars*) airway epithelial preparations to cumulative addition of forskolin (10^{-5} M) and IBMX (5 mM) in Ringer's solutions (A) or reduced luminal Cl^- solutions (B). Note change in scale of ordinate in panels A and B.

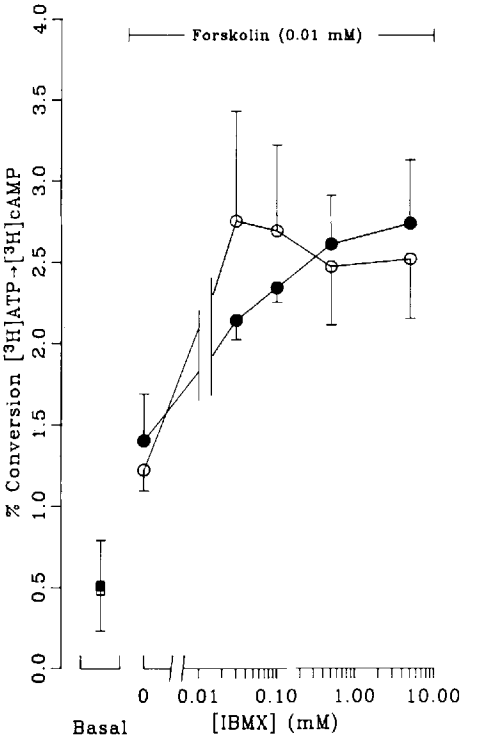

Figure 3. Effect of IBMX on forskolin-stimulated cAMP accumulation in human nasal epithelium. Cells from normal subjects (*solid symbols*) or CF patients (*open symbols*) were preincubated for 10 min with vehicle (basal) and with forskolin (10^{-5} M) alone or forskolin with the indicated concentration of IBMX. Squares denote basal cAMP levels for normal subjects (*solid square*) and CF patients (*open square*). Circles denote values obtained during forskolin treatment (see upper brackets) for normal subjects (*solid circles*) and CF patients (*open circles*). The results were plotted as the percent conversion of [^{3}H]cAMP and are the mean of triplicate determinations from four individuals.

the apical membrane P_2 receptor previously reported on these cells (25, 26). Importantly, the CF and normal cells both respond to ATP with Cl^- secretion, indicating that the lack of response to forskolin/IBMX in CF epithelia is not due to unresponsive tissues.

Because IBMX failed to increase the rate of Cl^- secretion of forskolin-treated normal and CF tissues (Figure 2), we tested whether IBMX in the range of concentrations employed was capable of maximizing the cellular cAMP levels in combination with forskolin. Results of the concentration-effect relationship between added concentrations of IBMX- and forskolin-stimulated cellular cAMP accumulation in epithelial cultures are shown in Figure 3. Forskolin induced an approximately 3-fold increase over basal cAMP levels. The addition of IBMX further raises the intracellular cAMP level, with a maximal effective concentration of 0.1 mM. No added effects are seen with 5 mM as compared with 0.1 mM. No differences were detected between CF and normal preparations.

We explored further the observation that IBMX inhibited I_{sc} after forskolin treatment in normal tissues. The inhibitory effect of IBMX on I_{sc} was similar when IBMX was delivered unilaterally on the basolateral or apical surface. The absolute magnitude of the inhibition of I_{sc} was directly proportional ($r = 0.79$, $P < 0.01$; $n = 11$) to the magnitude of the stimulation of I_{sc} by forskolin. The dose dependence of the inhibition was further investigated in forskolin-pretreated preparations that were sequentially exposed to 0.1 mM followed by 5 mM IBMX in the apical or basolateral solution. The forskolin-induced current (ΔI_{sc} = 12.5 ± 2.4 $\mu A \cdot cm^{-2}$) was not changed by the addition of 0.1 mM IBMX (0.5 ± 0.2 $\mu A \cdot cm^{-2}$), whereas the subsequent addition of 5 mM IBMX inhibited the current by 2.2 ± 0.5 $\mu A \cdot cm^{-2}$ ($n = 5$).

We performed two additional maneuvers in an attempt to stimulate Cl^- secretion in CF tissues *in vitro*. First, we took advantage of synergism of forskolin and isoproterenol to generate very high levels of intracellular cAMP (14). Although the combination of isoproterenol (10^{-5} M) and forskolin (10^{-5} M) was 3-fold more effective (36.7 ± 1.0% conversion of ATP to cAMP) in raising cell cAMP levels than forskolin (10^{-5} M) plus 0.1 mM IBMX (12.7 ± 3.4% conversion of ATP to cAMP), this combination did not induce Cl^- secretion in CF tissues (ΔI_{sc} = 0.1 ± 0.5 $\mu A \cdot cm^{-2}$, $n = 5$) and was no more effective in inducing Cl^- secretion in normal tissues than forskolin alone (ΔI_{sc} = 11.2 ± 1.2 $\mu A \cdot cm^{-2}$, $n = 3$). Second, we attempted to increase sensitivity by inhibiting possible dephosphorylation of CFTR using okadaic acid, a phosphatase inhibitor (27). Preliminary experiments suggested that 10^{-6} M okadaic acid was the maximal concentration that could be delivered without tissue damage. Pretreatment of normal tissues with okadaic acid (10^{-6} M) did not increase sensitivity to forskolin (ΔI_{sc} = 10.2± 2.1 $\mu A \cdot cm^{-2}$ and 14.6 ± 1.78 $\mu A \cdot cm^{-2}$ without and with okadaic acid, respectively; $n = 3$ each) nor did it unmask responses to forskolin and IBMX in CF tissues (ΔI_{sc} = 0.1 ± 0.8 $\mu A \cdot cm^{-2}$, $n = 4$). Similar findings were observed with another phosphatase inhibitor, calyculin A (28).

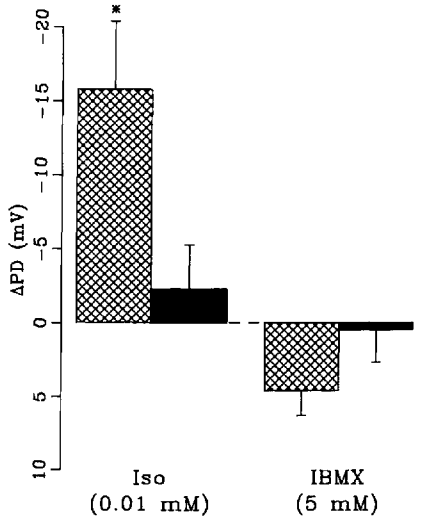

Figure 4. The change in nasal PD *in vivo* in normal subjects (*hatched bars*) and CF patients (*solid bars*) in response to cumulative addition of isoproterenol (10^{-5} M) and IBMX (5 mM) during superfusion with nominally Cl^--free solutions containing amiloride (10^{-4} M).

Finally, we tested whether IBMX could initiate Cl^- secretion *in vivo*. The nasal lumen was superfused with Cl^--free solutions containing amiloride to maximize sensitivity of the technique to small changes in apical membrane Cl^- conductance (22). Analysis of the steady-state responses revealed that isoproterenol induced a sustained (3 min) increase in PD in normal subjects and addition of IBMX had no further effect (Figure 4). In contrast, in CF patients no sustained responses were observed to isoproterenol or IBMX during up to 6 min of superfusion. ATP was effective in raising PD in both normal subjects ($\Delta PD = 11.3 \pm 3.2$ mV, $n = 3$) and CF patients ($\Delta PD = 14.1 \pm 2.1$ mV, $n = 3$), but the responses of both subject groups in the presence of 5 mM IBMX were smaller than previously reported (29).

Discussion

These studies demonstrate that high concentrations of IBMX (5 mM) combined with direct activation of adenylyl cyclase (forskolin) *in vitro* or a receptor-mediated adenylyl cyclase activator (isoproterenol) *in vivo* failed to stimulate Cl^- secretion in CF airway epithelia. The large Cl^- secretory responses of CF tissues to ATP, a Cl^- secretagogue of another class (25, 26), indicate that the tissues were capable of Cl^- secretion. The forskolin- or β-agonist-stimulated Cl^- secretory rates in normal tissues were not increased by IBMX.

Because forskolin, β-agonists, and IBMX modulate Cl^- secretory rates by a signal transduction system that involves cAMP, the relationships between these agents and cell cAMP levels were examined. Three key points emerged. First, based on the observation that forskolin alone is maximally effective in initiating Cl^- secretion (Figure 2), it appears that a 3-fold increase in cell cAMP levels (Figure 3) is sufficient to activate fully the cAMP-dependent pathway that regulates Cl^- secretion in human airway epithelia (30). This relationship is typical of other reports in which modest increases in cell cAMP levels were sufficient to activate fully cAMP-dependent effector pathways (31). Second, it appears that 0.1 mM IBMX in forskolin-pretreated normal and CF tissues generates the maximal concentration of cellular cAMP possible by this combination of reagents in human airway epithelial cells (Figure 3). Thus, the failure of the CF cells to respond to the combination of forskolin and IBMX (up to 5 mM) does not reflect a failure of these agents to achieve the maximal cellular cAMP concentrations possible with this drug combination. Third, the drug combination of isoproterenol and forskolin, operating synergistically on adenylyl cyclase (14), generates approximately 3-fold higher levels of cAMP than forskolin/IBMX but did not increase the magnitude of Cl^- secretory rates in normal tissues or induce Cl^- secretion in CF tissues. Thus, achieving cell cAMP levels substantially higher than possible with the combination of forskolin/IBMX does not activate Cl^- secretion in CF airway epithelia.

High concentrations of IBMX (5 mM) had unexpected effects on human airway epithelia. First, high concentrations of IBMX may block the Cl^- secretory path. Inhibition of I_{sc} in normal but not CF tissues is consistent with an inhibitory action of IBMX on the Cl^- secretory path. Because there is no difference in cellular cAMP levels in response to 0.1 and 5 mM of IBMX, this inhibitory effect likely is a nonspecific effect of IBMX. Second, IBMX at high concentrations (5 mM) appears to have weak antagonist effects on the purinergic receptor that transduces the ATP-induced Cl^- current.

The reasons for the discrepancy between the data presented here and those of Drumm and colleagues (11) are not clear but can be analyzed conceptually in several ways. The first analysis focuses on the cellular metabolism of ΔF508 CFTR and whether ΔF508 CFTR maintains partial Cl^- transport function. Recently, it has been suggested that the mutated CFTR (ΔF508) is recognized as abnormal and catabolized intracellularly before the insertion into the plasma membrane site required for Cl^- transport (32). It is conceivable that the overexpression of ΔF508 CFTR and/or perhaps inefficient intracellular catabolism of a foreign protein in frog oocytes permits partially functional (33) ΔF508 CFTR protein to be inserted in the oocyte membrane, where it is available for induction of cAMP-dependent Cl^- transport. The failure of forskolin and IBMX to stimulate endogenous ΔF508-mediated transport in airway epithelia could reflect the relatively low level of endogenous production of CFTR by airway epithelia (34) and/or efficient intracellular catabolism of ΔF508 CFTR in airway epithelia, resulting in little or no ΔF508 CFTR in the plasma membrane for cAMP-dependent Cl^- transport. A second possible explanation for the difference between our results and those of Drumm and colleagues (11) relates to the temperature at which the cells were cultured. It has been shown that 3T3 fibroblasts transfected with ΔF508 CFTR and maintained at 37°C failed to express a cAMP-mediated Cl^- conductance, whereas when these cells were maintained at 26°C, cAMP agonists induced a Cl^- conductance in them (35). Our airway epithelia were cultured at 37°C, whereas frog oocytes, which were em-

ployed in the study by Drumm and colleagues (11), are usually maintained at 26°C. The third possible difference between our study and that of Drumm's group (11) focuses on the level of phosphorylation of CFTR and its activity. It is conceivable that the degree of phosphorylation of CFTR is critical for activation of Cl^- transport (11), and that the ΔF508 CFTR endogenously expressed in airway epithelia cannot be phosphorylated to the same extent as the ΔF508 CFTR that is expressed in the frog oocyte system. The observation that Cl^- secretion in normal cells is maximally stimulated at less than maximal levels of cell cAMP indicates that the cellular phosphorylating activity (cAMP-dependent protein kinase) required for activation of Cl^- secretion in airway epithelia is fully maximized by forskolin alone. No comparative cellular cAMP data are available in the oocyte system. It is also conceivable that the phosphorylation of ΔF508 CFTR is lower in airway epithelia than in the oocyte because of relatively greater dephosphorylation (phosphatase) activity in airway epithelia. Although it is not yet known which cellular phosphatases are active on CFTR, treatment of airway epithelia with known permanent phosphatase inhibitors did not unmask effects of cAMP elevation on Cl^- secretion in CF airway epithelia.

In summary, the combination of activators of adenylyl cyclase and high concentrations of the phosphodiesterase inhibitor, IBMX, did not initiate Cl^- secretion in CF airway epithelia *in vitro* or *in vivo*. Thus, treatment of CF patients with high concentrations of methylxanthines, including theophylline, with the goal of inducing Cl^- secretion does not appear warranted. Indeed, IBMX at very high concentrations may have nonspecific effects, e.g., a block of Cl^- conductance and inhibition of P_2 receptors. In addition, it has been shown that agents that raise cell cAMP in CF airways may further increase the abnormally high basal rate of Na^+ transport (6). Thus, the development of Cl^- secretagogues in CF airway epithelia that focus on elevating the levels of cell cAMP concentrations may be unproductive, and strategies focusing on alternative pathways, such as purinoceptors, may be preferable.

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